IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Claudia ULBRICH et al.

Serial No.: 09/926,630

Filing Date: November 27, 2001

For: MEDICAMENTS FOR THE IMMUNOTHERAPY OF MALIGNANT TUMORS

RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371

5 KO 9/19/02

Box PCT
Commissioner for Patents
Washington, D.C. 20231

Attention: APPLICATION BRANCH
MISSING REQUIREMENTS OF APPLICATION

Sir:

A Notification of Missing Requirements, dated April 3, 2002, indicated that the following items are missing from the above-captioned application:

Translation of the application into English; and

Oath or Declaration.

However, Applicant respectfully submits that the Translation of the application into English was filed on November 27, 2001. The Declaration was filed on February 26, 2002. A copy of each paper filed is attached hereto, along with a copy of the date-stamped postcard evidencing receipt of these papers in the PTO on November 27, 2001 and February 26, 2002.

Appln. No.: 09/926,630

The Commissioner is hereby authorized to debit or credit any fees set forth in §1.16 or §1.17 to Deposit Account No. 06-1358 as needed in order to effect proper filing of the application. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By _____
William E. Player
Reg. No. 31,409

400 Seventh Street, N.W.
Washington, D.C. 20004-2201
(202) 638-6666

Atty. Docket No.: P67344US0
Date: June 3, 2002
WEP/cmf

US APPLICATION NO.(If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/EP01/08455	P67344US0

	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic Nati nal Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) . . $710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . $740.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) **$1040.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$890.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☒ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	**16** - 20 =	-0-	x $18.00	$	
Independent Claims	**2** - 3 =	-0-	x $84.00	$	
Multiple Dependent Claim(s) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1020.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 1020.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
TOTAL NATIONAL FEE =				$ 1020.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
TOTAL FEES ENCLOSED =				$ 1020.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ☒ A check in the amount of $ 1020.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 06-1358 in the amount of $ ____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. 06-1358. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

JACOBSON HOLMAN PLLC
400 7th Street, N.W., Suite 600
Washington, DC 20004
202-638-6666
CUSTOMER NUMBER: 00136

By _____
William E. Player
Reg. No. 31,409

JPH&S 3/95

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER P67344US0
	US APPLICATION NO.(If known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP01/08455	21 July 2001	28 July 2000

TITLE OF INVENTION

MEDICAMENTS FOR THE IMMUNOTHERAPY OF MALIGNANT TUMORS

APPLICANT(S) FOR DO/EO/US

Claudia ULBRICH, Klaus-Dieter ROCKENSUESS and Armin GROSSMANN

RECEIVED
AUG 26 2002
TECH CENTER 1600/2900

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    International Search Report - EPO
    PCT/IB/301 Form
    PCT/IB/304 Form

COPY

****HAND CARRY**** ***TO PCT WINDOW, CRYSTAL PLAZA II, 8TH FLOOR***

Atty Docket: P67344US0 Today's Date: November 27, 2001
Serial No.: New U.S. National Phase Application
Applicant: ULBRICH et al.
Filing Date: November 27, 2001

The following has been received in the U.S. Patent & Trademark Office on the date stamped hereon:

X Preliminary Amendment to Lessen Fees
X International Search Report (210)
X PCT/IB/301/304 Form(s)
X 11 pg. Specif. with 16 claims and Abstract

Check for $ 1020.00 Check No. 55668 **DUE DATE: ASAP**

09/926630

JACOBSON HOLMAN PLLC
400 SEVENTH STREET, NW
WASHINGTON, DC 20004

EARLY SERIAL NO. _____

WEP/cmf

BOX PCT

MISSING REQUIREMENTS OF APPLICATION
APPLICATION BRANCH

Today's Date: February 26, 2002

Att'y Docket No.: P67344US0
Serial No.: 09/926,630
Applicant: ULBRICH et al.
Filing Date: November 27, 2001

The following has been received in the U.S. Patent & Trademark Office on the date stamped hereon:

X Transmittal of Missing Requirements
X Combined Declaration, Power of Attorney

JACOBSON HOLMAN PLLC
400 SEVENTH STREET, NW
WASHINGTON, DC 20004

WEP/cmf

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Claudia ULBRICH et al.

Serial No.: 09/926,630

Filed: November 27, 2001

For: MEDICAMENTS FOR THE IMMUNOTHERAPY OF MALIGNANT TUMORS

TRANSMITTAL OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371

Box PCT
Commissioner for Patents
Washington, D.C. 20231

COPY

Attention: APPLICATION BRANCH
MISSING REQUIREMENTS OF APPLICATION

Sir:

With respect to the above-identified national phase application, the following are filed herewith.

___ Preliminary Amendment.

_X_ Declaration in compliance with 37 C.F.R. §1.63.

___ Declaration in compliance with 37 C.F.R. §1.63, attached to copy of specification as filed.

_X_ If a Petition for Extension of time is necessary and the Petition and/or the check is not enclosed, this will act as the Petition and applicant herewith petitions the Commissioner to extend the time for response and charge and fees necessary under 37 CFR 1.17 (a) - (d) to Deposit Account No. 06-1358. The Commissioner is also authorized to charge payment of any other additional fees associated with this communication or credit any overpayment to Deposit Account No. 06-1358. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By_____
William E. Player
Reg. No. 31,409

400 Seventh Street, N.W.
Washington, D.C. 20004-2201
(202) 638-6666

Atty. Docket: P67344US0
Date: February 26, 2002
HBJ/cmf

DECLARATION AND POWER OF ATTORNEY U.S.A.

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY; OR PROVISIONAL APPLICATIONS

FOR ATTORNEYS' USE ONLY

ATTORNEYS' DOCKET NO.

101 / 102

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

Medicaments for the immunotherapy of malignant tumors

which is described and claimed in:

[X] PCT International Application No. PCT/EP 01/08455 filed 21/07/2001

[ ] the attached specification

[ ] the specification in application Serial No. _____ filed _____

(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
00 116 362.5	Europe	28/07/2000	[X] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No

103 / 104

I hereby claim the benefit under Title 35, United States Code,§119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.) (Filing Date) (Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (22,772)

SEND CORRESPONDENCE TO: CUSTOMER NO. 00136 or	DIRECT TELEPHONE CALLS TO:
JACOBSON HOLMAN PROFESSIONAL LIMITED LIABILITY COMPANY 400 SEVENTH STREET, N.W. WASHINGTON, D.C. 20004	(please use Attorney's Docket No.) (202) 638-6666 **JACOBSON HOLMAN** PROFESSIONAL LIMITED LIABILITY COMPANY

*Inventor(s) name must include at least one unabbreviated first or middle name.

201	FULL NAME * OF INVENTOR	FAMILY NAME: Ulbrich	GIVEN NAME: Claudia	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Hannover	STATE OR FOREIGN COUNTRY: Germany	COUNTRY OF CITIZENSHIP: Germany	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Morgensternweg 15	CITY: Hannover	STATE OR COUNTRY: Germany	ZIP CODE: 30419
202	FULL NAME * OF INVENTOR	FAMILY NAME: Rockensüss	GIVEN NAME: Klaus-Dieter	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Fritzlar	STATE OR FOREIGN COUNTRY: Germany	COUNTRY OF CITIZENSHIP: Germany	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Am Hilgenstein 50	CITY: Fritzlar	STATE OR COUNTRY: Germany	ZIP CODE: 34560
203	FULL NAME * OF INVENTOR	FAMILY NAME: Grossmann	GIVEN NAME: Armin	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Hannover	STATE OR FOREIGN COUNTRY: Germany	COUNTRY OF CITIZENSHIP: Germany	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Jobstweg 8	CITY: Hannover	STATE OR COUNTRY: Germany	ZIP CODE: 30419

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
DATE 09/01/02	DATE 09/01/02	DATE 09/01/02

[ ] Additional inventors are named on separately numbered sheets attached hereto.

P67344US0

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents, Box PCT
United States Patent and Trademark Office
Washington, D.C. 20231
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
09/926,630	Claudia Ulbrich	P67344US0

INTERNATIONAL APPLICATION NO.	
PCT/EP01/08455	
I.A. FILING DATE	PRIORITY DATE
07/21/2001	

00136
JACOBSON HOLMAN PLLC
400 SEVENTH STREET N.W.
SUITE 600
WASHINGTON, DC 20004

JACOBSON HOLMAN PLLC
Response Due On Or Before
6 / 3 / 02
Month Day Year

CONFIRMATION NO. 1109
371 FORMALITIES LETTER

OC000000007762851

Date Mailed: 04/03/2002

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated Office (37 CFR 1.494):

- U.S. Basic National Fees
- Copy of the International Application
- Copy of the International Search Report
- English Translation of the IA
- Preliminary Amendments
- Request for Immediate Examination

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Translation of the application into English. The current translation of the application into English is defective as described below.
  - A copy of the Internation Application as filed and a copy of the Request Form 101
- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTH FROM THE DATE OF THIS NOTICE OR BY 22 or 32 MONTHS (where 37 CFR 1.495 applies) FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed

to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

*A copy of this notice **MUST** be returned with the response.*

CHARITTA A BURT

Telephone: (703) 305-3734

PART 1 - ATTORNEY/APPLICANT COPY

U.S. APPLICATION NUMBER NO.	INTERNATIONAL APPLICATION NO.	ATTY. DOCKET NO.
09/926,630	PCT/EP01/08455	P67344US0

FORM PCT/DO/EO/905 (371 Formalities Notice)

SMB

COPY

Medicaments for the Immunotherapy of Malignant Tumors

The present applications relate to compositions which are particularly suitable for the immunotherapy of malignant tumors, and methods for their preparation, and the use of the compositions for preparing medicaments.

Usually, the therapeutic treatment of tumors is effected by radical surgery, chemotherapy, radiotherapy or hormone therapy. These therapies have numerous undesirable side effects and are accompanied by significant loads on the patient. Moreover, in some tumor forms, almost no improvements are achieved with these therapies so that their use does not appear reasonable in view of the side effects. These forms include, in particular, malignant tumors, malignant melanomas, renal carcinomas, intestinal carcinomas and pancreatic carcinomas. Therefore, the mortality rate in, for example, renal carcinomas is 85%.

In recent years, knowledge has been increasingly gained on the complex interplay between tumors and the immune system, the interest becoming focused on strategies for treating tumors in which the immune system is stimulated. Generally, it is the object of such therapies to succeed in causing the immune system to recognize specific antigens from tumor cells which are not present in healthy cells, or only so to a lower extent. This is achieved, for example, by administering a medicament as described in Anticancer Research [(1997) No. 17, pages 2879-2882, and 3117-3120]: Tumor tissue is withdrawn from a patient and processed into an autologous tumor cell lysate, which is injected into the patient. This was done expecting that immunity against the tumor antigens is provided in the lysate. Another strategy is described in the published patent application WO-A 99/47687. It is disclosed therein that autologous antigen-presenting cells which express a special tumor determinant at their surfaces are injected into patients.

It is not only the object of tumor therapies to prevent the growth of tumors and the formation of metastases, but also to promote their regression. The patient's expectation of life is to be prolonged, and his health and life quality improved. About the success of immune therapies, it can be said at present that the therapeutic treatments used so far, unfortunately, can achieve success only in single cases or only in part. It is a basic problem that many tumor markers are also present in healthy cells in particular stages of differentiation and in certain amounts. Therefore, activation of the immune system against such tumor markers often does not occur to the extent desired or with the required specificity.

It has been the object of the present invention to develop medicaments for tumor therapy which achieve the above mentioned objects to a high extent. Also, when the medicaments according to the invention are used, it should be possible to perform tumor therapies relatively quickly and simply.

The present invention relates to a composition for the immunotherapy of tumors. The composition can be obtained by a process in which tumor material is evaluated, comminuted and transferred into a purified cell suspension, which is then incubated with interferon-gamma and tocopherol acetate and frozen to form a tumor cell lysate, and in which monocytes are isolated from buffy coats or whole blood and subsequently induced to differentiation into dendritic cells by incubation with cytokines and transferred into the non-adherent stage, whereupon a calculated amount of the above frozen tumor cell lysate is thawed, added as an antigen, cytokines are added, incubation is performed, and the mature dendritic cells produced are harvested.

"Evaluation" of the tumor material means macroscopic evaluation of the tissue, upon which clearly discernible proportions of adipose, connective and functional renal tissues, blood vessels and other non-tumor tissues are identified and subsequently removed and discarded.

In a particular embodiment, autologous tumor material is used for producing the composition. When the composition is produced, IL-4 and GM-CSF and/or IFN-gamma are preferably added to immature dendritic cells for differentiation.

The composition according to the invention is especially suitable as a medicament or for the preparation of a medicament for immunotherapy. Medicaments containing the cell lysate according to the invention are preferably injected intracutaneously or subcutaneously.

All conceivable types of solid tumor diseases can be treated with the medicament according to the invention. Medicaments containing the composition according to the invention are especially suitable for the treatment of tumors in which other treatment methods are little successful. In particular, in addition to other malignant solid tumors, these include malignant melanomas, renal carcinomas, intestinal carcinomas, pancreatic carcinomas, lymphomas, bronchial carcinomas and gynecological tumors.

When patients are treated with the medicament according to the invention within the scope of a tumor therapy, unexpectedly pronounced positive effects for the patients are observed. The growth of tumors and the formation of metastases could be prevented to a surprisingly high extent while the regression of tumors was promoted. The health, life quality and expectation of life of the patients were clearly increased. These effects can be achieved probably because the medicament according to the invention is distinct from known ones in essential aspects. One particular difference from many known methods is that tumor markers are not simply administered to the patient, but directly introduced into the patient's immune system in dendritic cells as vehicles. Surprisingly, it is sufficient to add a crude cell lysate of tumor cells to the dendritic cells in vitro, whereas according to WO-A-99/47687, antigen-presenting cells are admixed or transfected with a purified antigen. Therefore, the method according to the invention can also be performed more quickly and more simply as compared to known methods. This is especially important in the preparation of such therapeutic substances in order to keep the risk of contaminations low. In addition, the cell lysate has the advantage that the whole antigen repertoire of a tumor cell is available.

The present invention also relates to methods for the preparation of a medicament in which a suspension of tumor cells is prepared, the tumor cells are killed, and monocytes are isolated from blood, their differentiation into dendritic cells is

induced, and the thus obtained "immature" dendritic cells are incubated with the cell lysate of the killed tumor cells, the maturing of the dendritic cells is induced, and the "mature" dendritic cells are harvested.

The monocytes are preferably isolated from buffy coats, from separated stem cells, from leukapheretic products, or from whole blood.

The differentiation of the monocytes into "immature" dendritic cells is preferably induced by cytokines, IL-4 and GM-CSF. Especially suitable for induction of the maturing from "immature" to "mature" dendritic cells are prostaglandin E_2 and TNF-α and/or IL-1β and IL-6 in addition to IL-4 and GM-CSF. The preparation of the tumor cell suspensions is generally effected by isolating and optionally evaluating tumor material, which is then comminuted and transferred into a purified cell suspension. In a particular embodiment of the method according to the invention, the suspension of tumor cells is prepared from autologous tumor material. In another preferred embodiment, the expression of membrane-borne protein complexes is induced in the tumor cell suspension prior to said killing of the tumor cells. The induction is preferably effected by interferon-gamma and tocopherol acetate. The killing of the tumor cells is effected, in particular, by freezing. The harvesting of the mature dendritic cells is preferably performed when typical morphological characteristics are present (e.g., veil formation) as evaluated by microscopic check and/or by characterization of surface antigens using fluorescent antibodies. The invention also relates to the use of the described composition and its possible embodiments for preparing medicaments for tumor therapy.

According to the invention, the composition described and its possible embodiments are also used for the preparation of medicaments for tumor vaccination.

Example

Preparation of a composition for tumor therapy

A) Preparation of a tumor cell lysate

For preparing the tumor tissue, proportions of adipose, connective and functional renal tissues as well as blood vessels and necrotic tissues which are clearly discernible macroscopically are carefully removed and discarded. The ready prepared tissue is comminuted to a size as small as possible (pieces of about 2-3 mm diameter) and/or enucleated and then transferred into a sterile sieve (50-100 mesh) together with the surrounding medium. With a glass rod, a tissue pieces present in the sieve are passed through with slow stirring without pressure. The passed cells are transferred into a sterile beaker with medium RPMI 1640, and after addition of 15 ml of RPMI medium (RPMI 1640 with 25 mmol HEPES) into the sieve, the tissue remnants in the sieve are again passed through with a glass rod.

The cell suspension is layered onto 45% Percoll cushion. This step serves for the removal of any erythrocytes present and for the enrichment of mononuclear cells on the Percoll cushion. The filled tubes are centrifuged, and the interphase with the mononuclear cells is sucked off, transferred into a tube, pelletized by centrifugation and washed with NaCl/glucose solution. The total number of vital cells is determined microscopically using a Neubauer counting chamber after staining of the cells with trypan blue. In addition, cell typing is performed using TestSimplets® (Boehringer Mannheim), which are suitable for rendering carcinoma cells distinguishable from other cells in a quick staining process. After resuspension of the cells in sodium chloride/glucose solution, vitamin E (700 µg/dose to be prepared) and interferon-gamma (1500 IU/dose to be prepared) are added. The mixture is incubated in a water bath at 37 °C for two hours, centrifuged and washed twice with sodium chloride/glucose solution. The mixture is aliquoted into cryotubes and converted to a tumor cell lysate by freezing at –85 °C ± 5 °C. The quality controls comprise the tests according to specification for cell count, sterility and devitalization.

B) Preparation of the dendritic cells and of the composition for tumor therapy

Media employed:
Medium A: RPMI medium + 1% autologous plasma
Medium B: medium A + GM-CSF (800 U/ml) + IL-4 (1000 U/ml)
Medium C: medium B + TNF-α (1000 U/ml) + prostaglandin E_2 (1 µg/ml).

The buffy coats from released blood donations, from leukaphereses or whole blood from a blood bag are transferred into centrifuge tubes and centrifuged. The interphase contains the mononuclear cells (= buffy coat) and is separated from the erythrocytes (bottom) and the plasma (top). The plasma and mononuclear cells are layered on Lymphoprep® (Nycomed) and centrifuged. Subsequently, the plasma and mononuclear cells are pipetted off and again centrifuged. The plasma is taken off and used for preparing the media. Residual plasma is stored at from +2 °C to +8 °C in order to prepare additional medium A, if needed. The cell pellet is washed twice with NaCl solution (0.9%) and centrifuged. Prior to the second washing step, vital cells are counted after staining with trypan blue. The centrifugation residue is taken up in medium A at a cell concentration of 4×10^6/ml. The cell suspension is applied to Petri dishes and incubated at 37 °C ± 1 °C and 5% CO_2 for two hours. A microscopic check for adherent cells (monocytes) is then effected, whereupon medium A is carefully sucked off to remove non-adherent cells.

Medium B is added to the Petri dishes, followed by incubation at 37 °C ± 1 °C and 5% CO_2. On day 1, medium B is sucked off, and fresh medium is added. On day 2, medium B is sucked off partially (3 ml), and fresh medium B (3 ml) is added. On day 5, a microscopic check is effected to see whether adherent cells have undergone transition to the non-adherent stage. The cells of one charge are combined, and vital cells are counted after staining with trypan blue. The cells are centrifuged off and taken up in a calculated amount (5×10^5/well/3 ml) in medium C, the volume corresponding to one tenth of the final volume, a calculated amount of tumor cell lysate (5×10^4/well/3 ml) is added, followed by homogenization and incubation for one hour at 37 °C ± 1 °C and 5% CO_2, and then medium C is filled to the final volume. The cell suspension is plated on 6-well plates and further incubated at 37 °C ± 1 °C/5% CO_2. On days 6 and 7, a microscopic check is

performed: the maturing process of the dendritic cells starts to show by "veil formation". On day 8, the mature dendritic cells are "harvested" upon microscopic check when the "veil formation" has become pronounced. The mature dendritic cells are pelletized by centrifugation and washed twice. The centrifugation residue is taken up in 0.9% NaCl solution, vital cells are counted after staining with trypan blue, and 0.9% NaCl solution is used to adjust the desired cell count.

CLAIMS:

1. A composition obtainable by a process in which tumor material is evaluated, comminuted and transferred into a purified cell suspension, which is then incubated with interferon-gamma and tocopherol acetate and frozen to form a tumor cell lysate,

   and in which monocytes are isolated from buffy coats or whole blood and subsequently induced to differentiation into dendritic cells by incubation with cytokines and converted to the non-adherent stage,

   whereupon a calculated amount of the above frozen tumor cell lysate is thawed, added as an antigen, cytokines are added, incubation is performed, and the mature dendritic cells produced are harvested.

2. The composition according to claim 1, wherein autologous tumor material has been used for the preparation.

3. The composition according to claim 1, wherein IL-4 and GM-CSF are added for differentiation into "immature" dendritic cells in the preparation.

4. A medicament containing a composition according to at least one of claims 1 to 3.

5. A method for preparing a medicament in which a tumor cell a suspension of tumor cells is prepared, the tumor cells are killed, and monocytes are isolated from blood, their differentiation into dendritic cells is induced,

   and the thus obtained "immature" dendritic cells are incubated with the cell lysate of the killed tumor cells, the maturing of the dendritic cells is induced, and the "mature" dendritic cells are harvested.

6. The method according to claim 5, in which the monocytes are isolated from buffy coats, whole blood, leukaphereses, or separated stem cells.

7. The method according to claim 5 and/or 6, in which the differentiation of the monocytes into "immature" dendritic cells by cytokines, IL-4 and GM-CSF with or without interferon-gamma.

8. The method according to at least one of claims 5 to 7, in which the maturing from "immature" to "mature" dendritic cells is induced by prostaglandin E_2 and TNF-α and/or IL-1β and IL-6 in addition to IL-4 and GM-CSF.

9. The method according to at least one of claims 5 to 8, in which the tumor cell suspension is prepared by isolating and optionally evaluating tumor material, which is then comminuted and transferred into a purified cell suspension.

10. The method according to at least one of claims 5 to 9, in which the tumor cell suspension is prepared by isolating and optionally evaluating autologous tumor material, which is then comminuted and transferred into a purified cell suspension.

11. The method according to at least one of claims 5 to 10, in which the expression of membrane-borne protein complexes is induced in the tumor cell suspension prior to said killing of the tumor cells.

12. The method according to claim 11, in which the expression of membrane-borne protein complexes is induced by interferon-gamma and tocopherol acetate.

13. The method according to claim 5, in which the tumor cells are killed by freezing.

14. The method according to claim 5, in which the "mature" dendritic cells are harvested when typical morphological characteristics are present (e.g., veil

formation) as evaluated by microscopic check and/or by characterization of surface antigens using fluorescent antibodies.

15. Use of the composition according to claim 1 for preparing a medicament for tumor therapy.

16. Use of the composition according to claim 1 for preparing a medicament for tumor vaccination.

Abstract

The present invention relates to a composition which can be used as a medicament or for preparing a medicament for the immunotherapy of tumors of for tumor vaccination.

The invention also relates to methods for preparing medicaments for the immunotherapy of tumors of for tumor vaccination.